

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule encoding a isoprenoid biosynthetic enzyme, selected from the group consisting of:
  - 5 (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18 and 24;
  - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS  
10 followed by 0.1X SSC, 0.1% SDS; and
  - (c) an isolated nucleic acid molecule that is complementary to (a) or (b).
2. The isolated nucleic acid molecule of Claim 1 selected from the  
15 group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17 and 23.
3. A polypeptide encoded by the isolated nucleic acid molecule of Claim 1.
4. The polypeptide of Claim 3 selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, and 18.
- 20 5. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 620 amino acids that has at least 60% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:2 or a second nucleotide sequence comprising the complement of  
25 the first nucleotide sequence.
6. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 394 amino acids that has at least 55% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ  
30 ID NO:4 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
7. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 231 amino acids that has at least 52% identity based on the Smith-Waterman method of alignment  
35 when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

8. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 285 amino acids that has at least 50% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:8 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

9. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 157 amino acids that has at least 69% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:10 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

10. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 544 amino acids that has at least 67% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:12 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

11. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 297 amino acids that has at least 57% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:14 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

12. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 511 amino acids that has at least 34% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:16 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

13. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 497 amino acids that has at least 49% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:18 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

14. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 318 amino acids that has at least 65% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:24 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

15. A chimeric gene comprising the isolated nucleic acid molecule of any one of Claims 1 or 5-14 operably linked to suitable regulatory sequences.

16. A transformed host cell comprising the chimeric gene of Claim 15.

17. The transformed host cell of Claim 16 wherein the host cell is selected from the group consisting of bacteria, yeast, filamentous fungi, and green plants.

18. The transformed host cell of Claim 17 wherein the host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Streptomyces*, *Escherichia*, *Pseudomonas*, *Methylobacter*, *Alcaligenes*, *Synechocystis*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and *Klebsiella*.

19. The transformed host cell of Claim 17 wherein the host cell is selected from the group consisting of soybean, rapeseed, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops, sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees, and forage grasses.

20. A method of obtaining a nucleic acid molecule encoding an isoprenoid compound biosynthetic enzyme comprising:

- (a) probing a genomic library with the nucleic acid molecule of any one of Claims 1 or 5-14;
- (b) identifying a DNA clone that hybridizes with the nucleic acid molecule of any one of Claims 1 or 5-14; and
- (c) sequencing the genomic fragment that comprises the clone identified in step (b),

wherein the sequenced genomic fragment encodes an isoprenoid biosynthetic enzyme.

21. A method of obtaining a nucleic acid molecule encoding an isoprenoid biosynthetic enzyme comprising:

- 5 (a) synthesizing an at least one oligonucleotide primer corresponding to a portion of the sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17 and 23; and
- (b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a);

10 wherein the amplified insert encodes a portion of an amino acid sequence encoding an isoprenoid biosynthetic enzyme.

22. The product of the method of Claims 20 or 21.

23. A method for the production of isoprenoid compounds comprising: contacting a transformed host cell under suitable growth conditions with an effective amount of a carbon source whereby an isoprenoid compound is produced, said transformed host cell comprising a set of nucleic acid molecules encoding SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18 and 24 under the control of suitable regulatory sequences.

24. A method according to Claim 23 wherein the transformed host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Streptomyces*, *Escherichia*, *Pseudomonas*, *Methylobacter*, *Alcaligenes*, *Synechocystis*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and *Klebsiella*.

25. A method according to Claim 23 wherein said methanotrophic bacteria:

- 25 (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
- (b) comprises a functional Embden-Meyerof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme.

26. A method according to Claim 25 wherein said methanotrophic bacteria is *Methylobacter* 16a ATCC PTA 2402.

27. A method according to Claim 23 wherein the transformed host cell is selected from the group consisting of soybean, rapeseed, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops,

sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees, and forage grasses.

28. A method according to Claim 23 wherein the carbon source is selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, carbon dioxide, methanol, methane, formaldehyde, formate, and carbon-containing amines.

29. A method according to Claim 23 wherein the transformed host is selected from the group consisting of *Methylomonas*, *Methylobacter* and *Methanobacterium* and the carbon source is selected from the group consisting of methane and methanol.

30. A method of regulating isoprenoid biosynthesis in an organism comprising, over-expressing at least one isoprenoid gene selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17 and 23 in an organism such that the isoprenoid biosynthesis is altered in the organism.

31. A method according to Claim 30 wherein said isoprenoid gene is over-expressed on a multicopy plasmid.

32. A method according to Claim 30 wherein said isoprenoid gene is operably linked to an inducible or regulated promoter.

33. A method according to Claim 30 wherein said isoprenoid gene is expressed in antisense orientation.

34. A method according to Claim 30 wherein said isoprenoid gene is disrupted by insertion of foreign DNA into the coding region.

35. A mutated gene encoding a isoprenoid enzyme having an altered biological activity produced by a method comprising the steps of:

- (i) digesting a mixture of nucleotide sequences with restriction endonucleases wherein said mixture comprises:
  - a) a native isoprenoid gene;
  - b) a first population of nucleotide fragments which will hybridize to said native isoprenoid gene;
  - c) a second population of nucleotide fragments which will not hybridize to said native isoprenoid gene;

wherein a mixture of restriction fragments are produced;

- (ii) denaturing said mixture of restriction fragments;
- (iii) incubating the denatured said mixture of restriction fragments of step (ii) with a polymerase;

- (iv) repeating steps (ii) and (iii) wherein a mutated isoprenoid gene is produced encoding a protein having an altered biological activity.